Influence of CD40 rs1883832 Polymorphism in Susceptibility to and Clinical Manifestations of Biopsy-proven Giant Cell Arteritis

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ABSTRACT. Objective. To assess the potential association between CD40 rs1883832 polymorphism and biopsy-proven giant cell arteritis (GCA). We also studied the influence of the polymorphism on phenotypic expression of this vasculitis, in particular the development of visual ischemic manifestations.

Methods. Three hundred five Spanish patients with biopsy-proven GCA and 788 matched controls were assessed. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for the CD40 rs1883832 C/T polymorphism using a predesigned TaqMan allele discrimination assay and by polymerase chain reaction amplification.

Results. Patients with GCA showed a trend toward a higher frequency of the minor allele homozygote of rs1883832 (TT) compared to healthy controls (12.1% vs 8.3%, respectively; p = 0.05, OR 1.54, 95% CI 0.98–2.40). Also, a marginally significant increased frequency of the minor allele T was observed in patients with GCA who had visual ischemic manifestations (27.7%; p = 0.04, OR 1.53, 95% CI 0.99–2.34). In this regard, patients with GCA carrying the minor allele T (either TT or TC) experienced visual ischemic manifestations more commonly than those carrying the CC genotype (58.5% vs 44.2%; p = 0.04, OR 1.78, 95% CI 0.99–3.22).

Conclusion. Our results suggest a potential implication of the CD40 rs1883832 C/T polymorphism in susceptibility to visual ischemic manifestations in individuals with biopsy-proven GCA.

Key Indexing Terms: GIANT CELL ARTERITIS TEMPORAL ARTERY BIOPSY GENETICS CD40 GENE POLYMORPHISM RS1883832 VISUAL ISCHEMIC MANIFESTATIONS

Giant cell arteritis (GCA) is the most common type of systemic vasculitis in Western countries in individuals over age 50. The immune attack, affecting medium-size and large arteries, leads to damage of the wall structures and to rapid concentric hyperplasia of the intima, followed by luminal occlusion. Clinical manifestations reflect end-organ ischemia, including blindness, jaw claudication, or stroke. GCA is a complex polygenic disease. Various gene polymorphisms have been associated with either disease susceptibility or a higher risk of severe ischemic complications.
GCA inflammatory lesions infiltrate all layers of the arterial wall, and are composed of T cells, dendritic cells (DC), highly activated macrophages, and in some cases multinucleated giant cells. However, B cell infiltration is rare.

CD40 is a type I transmembrane protein receptor of the tumor necrosis factor (TNF) superfamily. Activation of CD40 results in binding to TNF receptor-associated factors and upregulation of proinflammatory genes. Various studies support an important role of this moiety in vascular wall inflammation: CD40 is constitutively expressed in vascular wall cells such as endothelial cells (EC) and smooth muscle cells (SMC), macrophages, DC, and fibroblasts. CD40/CD40L interactions on the EC result in endothelium and SMC activation and expression of adhesion molecules, promoting leukocyte recruitment and migration into tunica media. CD40 upregulates expression of local vascular endothelial growth factor and basic fibroblast growth factor, promoting in vivo angiogenesis (a major structural alteration in the inflamed tunica media is the formation of blood vessels). CD40/CD40L has a role in DC/T cell interactions inside the vascular wall. CD40L on activated T cells interacts with CD40 on DC, enhancing several costimulatory ligands on the DC that interact with T cell costimulatory receptors, promoting a positive feedback loop that drives differentiation. CD40 is a major mechanism involved in interleukin 12 (IL-12) production by DC. In turn, IL-12 is dominant in directing the development of naive CD4 T cells into T helper (Th)1 cells that produce high amounts of interferon-γ (IFN-γ). IFN-γ tissue concentration correlates with the degree of intimal thickening, the extent of neovascularization, and the formation of multi-nucleated giant cells.

There is a single-nucleotide polymorphism located in the 5' untranslated region (Kozak sequence) of the CD40 gene (−1C/T, rs1883832). Its major allele has been associated to Graves' disease. Also, the major allele of another CD40 polymorphism (rs4810485), in almost complete linkage disequilibrium with rs1883832 (r² = 0.95), has been associated with rheumatoid arthritis (RA).

The CD40 rs1883832 major allele has been associated with an increased translational efficiency of nascent CD40 mRNA transcripts, resulting in an increase of CD40 expression at the cell surface. CD40 is known to be a regulator of retinal inflammation and neurovascular degeneration. However, to our knowledge, no previous studies have linked the CD40 rs1883832 polymorphism with ophthalmological diseases. Taking into account this evidence, we aimed to assess the potential association between the rs1883832 CD40 polymorphism and biopsy-proven GCA. We also studied whether this polymorphism might influence the phenotypic expression of this vasculitis, in particular the development of visual ischemic manifestations.

RESULTS

The median age at the time of disease diagnosis in this series of 305 patients with biopsy-proven GCA was 75 years (interquartile range 70-79 yrs). Women (n = 209; 68.5%) outnumbered men. Headache was the most common feature (n = 243; 79.7%). An abnormal temporal artery on physical examination was observed in 168 (55.1%) patients. Also, 146 (47.9%) had PMR. Jaw claudication occurred in 127 (41.6%). Visual ischemic manifestations were observed in 65 (21.3%) patients. Fourteen (4.6%) experienced a stroke. Severe ischemic complications (defined if at least 1 of the following was observed: visual ischemic manifestations, cerebrovascular accidents, jaw claudication, or limb claudication of recent onset) were found in 163 (53.4%) patients.

No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. The case:control ratio was 1:2.5. The power of this study for finding a difference between patients with GCA and healthy controls was...
between 69% and 98%, with an estimated OR between 1.5 and 2.0, a type I error rate of 0.05, a dominant inheritance mode and 0.0001% of population risk.

Influence of CD40 rs1883832 polymorphism in the susceptibility to GCA. Patients with GCA showed a trend toward a higher frequency of the minor allele homozygote of rs1883832 (TT) compared to controls (12.1% vs 8.3%, respectively; p = 0.05, OR 1.54, 95% CI 0.98–2.40; Table 1). In this regard, the frequency of the minor allele T was increased among patients with GCA compared to controls but the difference did not achieve statistical significance (29.7% vs 27.0%, respectively; p = 0.20).

Genotype and allele frequencies of CD40 rs1883832 polymorphism according to patients’ clinical manifestations. To further investigate the potential role of the CD40 rs1883832 polymorphism in the phenotypic expression of this vasculitis, patients with GCA were stratified according to the occurrence of PMR, visual ischemic complications, or severe ischemic manifestations, and then assessed for the allele and genotype distribution (Table 2). No significant differences were found in the allele or genotype frequencies between patients with GCA, either with or without PMR (Table 2). However, a marginally significant increased frequency of the minor allele T was observed in patients who had visual ischemic manifestations (36.9%) compared to patients who did not have visual ischemic manifestations (27.7%; p = 0.05, OR 1.54, 95% CI 0.99–2.40). However, the correction of p value for the number of alleles tested yielded a p value for the allele T association with visual ischemic manifestations slightly out of the range of significance (p = 0.08).

Also, patients with GCA carrying the minor allele T (either TT or TC) experienced visual ischemic manifestations more commonly than those carrying the CC genotype (58.5% vs 44.2%, respectively; p = 0.04, OR 1.78, 95% CI 0.99–3.22). Moreover, there was a nonsignificant trend toward a higher frequency of the minor allele T among patients with severe ischemic complications (32.5%) compared to those without these complications (26.4%; p = 0.10, OR 1.34, 95% CI 0.93–1.94). In this regard, the frequency of individuals carrying the minor allele T was increased among patients who had severe ischemic complications (52.1%) compared to those without severe ischemic complications (41.5%) but the difference remained slightly out of the range of significance (p = 0.065, OR 1.53, 95% CI 0.95–2.48).

Comparison of patients with GCA according to the presence or absence of other clinical manifestations did not yield statistically significant differences (data not shown).

DISCUSSION

We analyzed for the first time the potential implication of the CD40 rs1883832 C/T polymorphism in susceptibility to biopsy-proven GCA. We observed a nonsignificant trend for association between the genotype TT (homozygous for the minor allele T) and biopsy-proven GCA. However, differences in allelic frequencies between patients with GCA and healthy controls were smaller. Our data, assessing the largest series of GCA included in a genetic study, also disclosed a marginally significant increased frequency of the minor allele T of the CD40 rs1883832 polymorphism in the subgroup of patients with GCA who experienced visual ischemic manifestations. We also observed a higher frequency of the minor allele T among patients with severe ischemic complications, but the differences were smaller and did not reach statistical significance, probably because of an insufficient sample size, as GCA is a relatively uncommon disease.

The CD40 rs1883832 C/T polymorphism has previously been associated with Graves’ disease24,37 and multiple sclerosis38. Another CD40 polymorphism (rs4810485) in linkage disequilibrium with rs1883832 (r2 = 0.95) has been associated with an increased risk for RA25 and a higher rate of joint destruction in patients with this chronic inflammatory rheumatic disease39. In both Graves’ disease and RA the allele associated with a higher risk of disease susceptibility or with worse outcome was the major allele C. This major allele C has been associated with a higher expression of the CD40 moiety in cell surface of peripheral blood mononuclear cells, B cells, and platelets26,27,40. However, in keeping with data reported in patients with multiple sclerosis, in our series of GCA the allele that seems to be associated with a worse outcome (manifested by increased risk of visual ischemic complications) was the minor allele T.

As discussed, arterial wall inflammation leads to rapid concentric hyperplasia of the intima, followed by luminal occlusion in patients with GCA3,4. Because of the potential role played by CD40/CD40L interaction in the stimulus of IL-12 secretion by local DC, it is possible that the smaller number of CD40 moieties on the cell surface associated with the minor allele T of the rs1883832 polymorphism26 might predispose to a higher risk of visual ischemic manifestations in patients with GCA. IL-12 is dominant in directing the development of naive CD4+T cells into Th1 cells that produce high amounts of IFN-γ, a key cytokine in GCA, whose tissue concentration correlates with the degree of intimal thickening, the extent of neovascularization, and the
formation of multinucleated giant cells. Moreover, the source of IL-12 is highly restricted to DC that produce this cytokine after stimulation with either bacterial components such as lipopolysaccharide (LPS) or during the interaction with CD4+ T cells, because of the ligation of either CD40 or MHC class II molecules on DC. LPS-induced IL-12 plays an important role in the activation of the effector mechanisms in the initial phase of immune response in infected tissues, initiating an innate resistance to the pathogen while ensuring induction of the correct class of adaptive host response. However, the major mechanisms involved in IL-12 induction appear to be signaling through DC surface CD40 molecules or MHC class II molecules. Considering that GCA has been proposed to be an antigen-driven disease, we hypothesize that CD40 rs1883832 C/T polymorphism might contribute to an insufficient initial immune reaction against the antigen or antigens responsible for this vasculitis, leading to a situation in which the antigen cannot be completely eradicated, and chronically stimulates the immune system inside the arterial wall. This hypothesis agrees with previous observations in which patients with biopsy-proven GCA who suffered visual ischemic manifestations were associated with an initial lower inflammatory response. The fact that subjects not carrying CD40 rs1883832 minor allele T develop this condition may be due to the intrinsic characteristics of the antigen responsible for the disease, that is, it is difficult to eradicate completely even with a normal amount of CD40 in the cell surface.

Based on our data, the presence of this genetic variant could help to identify patients with biopsy-proven GCA who have a higher risk of a worse visual outcome. Further studies in large series of patients with GCA are needed to confirm our observations.

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